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Effect of integrated nutrient management on soil enzymes, microbial biomass carbon and bacterial populations under rice (*Oryza sativa*) –wheat (*Triticum aestivum*) sequence

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ABSTRACT

Key soil enzymes, microbial biomass carbon (MBC) and bacterial population were assessed to gain understanding the effects of integrated nutrient management (INM) under rice–wheat sequence grown during 2008 and 2009 in acid soils of Asom. Among the organic inputs, enriched compost (2 tonnes/ha) application demonstrated clear increase in Fluorescein di-acetate (FDA) hydrolase (8.91µg fluorescein/g/h), phosphomonoesterase (PMEase) (358.62 µg *p*-nitro phenol/g/h) and dehydrogenase (DH) (177.11 µg TPF/g/24 h) activities with only 25% of recommended N and P fertilizer under the sequence followed by compost (2 tonnes/ha) with biofertilizer inoculation. Likewise, highest MBC (183.66 µg/g) and maximum organic carbon (OC) (10.38 g/kg) accretion were obtained in the treatment received enriched compost (2 tonnes/ha) continuously for four crops. Compost (2 tonnes/ha) coupled with biofertilizers resulted maximum *Azospirillum* (5.79 log cfu/g), whereas enriched compost (2 tonnes/ha) favored elevated phosphate-solubilizing bacterial (PSB) (5.10 log cfu/g) population in the study. Significant correlations were existed among the enzymes as well as with buildup MBC, OC, *Azospirillum* and PSB population under the sequence. Application of compost (2 tonnes/ha) and biofertilizers with 25% recommended N and P fertilizer resulted significant increase in available N (234.11 kg/ha). Similarly, rock phosphate (RP) carrying enriched compost showed highest P (28.04 kg/ha) status of the soils. The overall multifaceted effects of different INM treatments that facilitated beneficial soil conditions in the present study reflected the significant increasing the grain yields of both rice (3.68 tonnes/ha) and wheat (0.98 tonnes /ha) even over the 100% NPK.

Key words: Fluorescein di-acetate, Dehydrogenase, Microbial biomass carbon, Phosphomonoesterase

Soil properties based on biological and biochemical activities, especially those involved in energy flow and nutrient cycling, have been shown to respond to small changes in soil conditions, thus providing information sensitive to subtle alterations of soil quality (Pascual *et al.* 2000). The soil microbial biomass is fundamental to maintaining soil functions because it represents the main source of soil enzymes that regulate transformation processes of elements in soils. Soil enzyme activities have been proposed as appropriate indicators because of their intimate relationship to soil biology, and rapid response to change in soil management. Suite of soil enzyme activities that integrate both the intra- and extra-cellular bio-geochemical activities of the soil biological system are the key aspects of soil quality assessment. Fluorescein di-acetate is hydrolyzed by a number of different enzymes, such as proteases, lipases, and esterases to produce fluorescent compound fluorescein and provide comprehensive microbial activity. Phosphomonoesterase is a generic name for a group of enzymes which catalyse the hydrolysis of esters of phosphoric acid in releasing phosphate and is of paramount importance as a soil quality indicator (Trasar-Cepeda *et al.* 2008). Dehydrogenase exist as an integral part of intact cells, involved in oxidative phosphorylation, and reflect the total oxidative potential of the soil microbial community (Dick 1997).

The objective of this study was therefore to investigate how INM have affected microbial biomass, enzyme activity, and the specific bacterial population under rice–wheat sequence. Among the different crop sequences, rice–wheat is one of the sequences cultivated in Asom. Despite the increasing trend of fertilizer consumption (58.60 kg/ha), the productivity of rice (1 446 kg/ha) and wheat (1 010 kg/ha) are far below the national average. Use of imbalance chemical

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fertilizer without proper organic amendments could have reduced the fertility status of soil. Measuring soil microbial biomass and enzymatic catalysis are the primary phase in characterizing soil metabolic potential, fertility and quality, as well as to guide to the resilience of the soil, since they can anticipate changes in soil quality before they are detected by other soil analyses (Ndiaye *et al.* 2000).

MATERIALS AND METHODS

Field experiment on rice–wheat sequence was conducted at Instructional Cum Research (ICR) farm of Assam Agricultural University for two years during 2008 and 2009. The experiment was laid down in randomized block design replicated thrice with eight treatment combinations. The plot size was 3m × 4 m. The eight treatments consisted of absolute control (T_1), 100% recommended doses (RD) of inorganic NPK (T_2), 50% RD of inorganic NP+100% K+ biofertilizers (T_3), 50% RD of inorganic NP+100% K+ biofertilizers+ compost @ 1tonne/ha(T_4), 25% RD of inorganic NP+100% K+ biofertilizers+ compost@ 2tonnes/ha(T_5), 50% RD of inorganic NP+100% K+ enriched compost @ 1tonne/ha (T_6), 25% RD of inorganic NP+100% K+ enriched compost @ 2tonnes/ha (T_7) and biofertilizers + compost@1 tonne/ha(T_8). The RD of fertilizers were 40:20:20 and 80:46:42 ($N:P_2O_5:K_2O$ kg/ha) for rice and wheat, respectively. The N, P and K were supplied through urea, single superphosphate and muriate of potash, respectively. Full doses of P and K were applied as basal, while N applied in three splits (50% as basal, 25% at tillering and 25% at panicle initiation) in rice. For wheat, 50% of N and entire quantity of P and K were applied as basal and remaining 50% of N at CRI stage. Two irrigations, first at CRI and second at heading stage were applied in wheat. Biofertilizers for rice (*Azospirillum* and phosphate-solubilizing bacteria (PSB), each @ 3.5 kg/ha) were applied as seedling dip and for wheat (*Azospirillum* and PSB, each @ 500 g for 10 kg seeds) as seed coating. The compost and enriched compost (primed with *Azospirillum* and PSB @1% broth each containing 10^8–10^9 cfu/ml and adjusted with 1% RP(as P) and cured for 1 month) used in the experiment was prepared from rice biomass. The *p*H of the experimental site was 4.70 with 9.6 g/kg OC and available N, P and K were 200.70, 21.91 and 146.07 kg/ha respectively. Rice (var. Ranjit) was transplanted during second week of June and wheat (var. PBW 343) was sown third week of November every year. The soil samples were drawn after harvest of each crop for both the years. OC, *p*H, available N, P and K were analyzed as per the method described by Jackson (1973). Prior to estimation of soil enzymatic activities and MBC the field moist samples were preserved in refrigerator at 4^0C.

FDA hydrolysis activities were carried out following the method described by Adam and Duncan (2001). Soil was incubated with the substrate, FDA, at 25^0C for 1hr. The amount of fluorescein formed was determined colorimetrically (Nano Drop 1000 spectrophotometer) following extraction with an organic solvent mixture (2:1 chloroform: methanol). Using the calibration curve the mass of fluorescein produced in each assay from the corresponding optical density (OD at 490nm) value was calculated on dry weight basis. Fluorescein diacetate hydrolysis activity was expressed as μg fluorescein/g dry soil/hr.

DH activities were determined by the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) as described by Casida *et al.* (1964) with modifications. Briefly, field moist soil (1 g) was treated with 1 ml of 3% TTC, and then incubated at 28°C for 24 hr. To account for any abiotic TTC reductions, sterile controls consisted of autoclaved soil (121°C, 20 min. on three consecutive days) were used. Spectrophotometer blanks for both autoclaved and non-autoclaved treatments consisted of soil and TTC replaced with millipore water. Controls and blanks treated like samples. The optical density at 485 nm was compared to that of triphenyl formazan standards. DH activity was expressed on dry weight as μg TPF/g dry soil /24hr.

PMEase activities involve the use of an artificial substrate, *p*-nitrophenyl phosphate (*p*-NPP).The product of Phosphomonoesterase activity, *p*-nitrophenol, a chromophore under alkaline conditions were detected colorimetrically following the method of Tabatabi and Bremner (1969) and expressed as μg *p*-nitrophenol/g dry soil/hr.

MBC was determined by chloroform fumigation extraction technique following the method of Vance *et al.* (1987). Field moist soil (25 g) was fumigated with ethanol free chloroform at 25 °C for 24hr. After fumigation, chloroform vapors were removed by repeated evacuation. The soil samples were then extracted with 100 ml 0.5M K_2SO_4 (1:4 soil: K_2SO_4). Controls were prepared by extracting soils without fumigation. The soil suspension was filtered through Whatman No 42 filter paper. Total OC content in the soil extract was estimated with dichromate (66.7mM) digestion method (Walkley and Black 1934). MBC was calculated from the differences in extractable OC between the fumigated and non-fumigated soil and expressed as μg/g dry soil.

The classical serial dilution technique was used for enumeration of *Azospirillum* and PSB by spread plate technique on nitrogen-free bromothymol blue (NFb) and Pikovskaya's solid media respectively. Rhizosphere soil sample of 1g was suspended in 9 ml water blank, followed by serially diluted up to 10^4 and 10^5. Aliquots of 100μl of 10^4 and 10^5 dilutions were spread over the solidified media in triplicates and plates were incubated at 30±1°C for PSB while NFb plates were incubated at 35±1°C for 3days. The microbial numbers were estimated as colony forming unit (cfu/g)/gram soil on dry weight basis and transformed to log cfu/g.

The entire datasets after four crops were pooled (for rice and wheat, two crops each) and analyzed using statistical package CPCS1 (1.0). Simple correlation coefficient between soil chemical properties and enzymatic activities were analyzed to establish possible statistical relationship (Chandel 2004).

RESULTS AND DISCUSSION

Soil enzymes

Fluorescein extractions under different INM treatments at increasing biological activity are shown in Table 1. The INM treatments influenced the overall level of FDA hydrolysis notably. FDA hydrolysis has been suggested as a measure of the global hydrolytic capacity of soils and a broad spectrum indicator of soil biological activity. There were consistent trend in FDA hydrolysis observed under the treatments that significantly differ from the control. The fluorescein produced in the treatments of T_5, T_6 and T_7 after the harvest of four crops under rice–wheat sequence were significantly higher (8.83–8.91 µg fluorescein/g/hr) even over the recommended dose of chemical fertilizer (7.71µg fluorescein/g/h).These significant increases of FDA hydrolysis was either due to the combined use of compost and biofertilizers or enriched compost with substantial reduction of inorganic fertilizer. The maximum increase in FDA hydrolysis (8.91 µg fluorescein /g/hr) was recorded in the treatment $T_{7,}$ which received enriched compost (2 tonnes/ha) with only 25% of NP fertilizer. The large increase of FDA hydrolysis due to combined use of enriched compost and fertilizers could be attributed to increased microbial biomass resulting from continuous organic matter enrichment in soil, since addition of good quality compost have direct bearing on MBC and soil enzyme activities (Singh *et al.* 2009b, Singh and Dhar 2011). FDA hydrolase showed significant correlation with MBC, OC, *Azospirillum* and PSB population (Table 2). Nayak *et al.* (2007) similarly demonstrated enhanced FDA hydrolysis activity with both compost and inorganic fertilizer under continuous rice-growing situation and showed significant relationship with OC. PMEase is an enzyme of agronomic value because it hydrolyses compounds of organic P and transforms them into inorganic P. The PMEase activity was lowest (280.64 µg *p*-nitro phenol/g/hr) in control plots that obtained no fertilizer or organic inputs (Table 1). Application of enriched compost or conjoint use of biofertilizers and compost with reduction of inorganic N and P fertilizers in treatments T_4, T_5, T_6 and T_7 demonstrated significantly higher PMEase activity (312.91–358.62 µg *p*-nitro phenol/g/hr) over the control (280.64 µg *p*-nitro phenol/g/h).Steady application of enriched compost (2 tonnes/ha) for four crops in the

Table 1 Soil enzymes activities, microbial biomass carbon, *and Azospirillum* and PSB population under INM in rice–wheat sequence

Treatment	FDA activity (µg fluorescein/g/h)	PMEase activity (µg p-nitro phenol/g/ h)	DH activity (µg TPF/ g/ 24h)	MBC (µg /g)	*Azospirillum* (log cfu/g)	PSB (log cfu/ g)
T_1	6.24	280.64	56.76	43.23	4.90	4.09
T_2	7.71	300.27	64.63	49.60	5.22	4.31
T_3	7.34	308.14	90.42	73.34	5.32	4.58
T_4	7.07	312.91	90.29	76.11	5.43	4.69
T_5	8.83	331.79	152.94	167.66	5.79	4.78
T_6	8.88	315.59	132.57	112.80	5.39	4.72
T_7	8.91	358.62	177.11	183.66	5.68	5.10
T_8	6.59	306.93	94.84	61.03	5.26	4.23
CD (P=0.05)	0.56	32.36	17.83	9.29	0.29	0.46

Means of pooled analysis after the four crops under the sequence

Table 2 Simple correlation between soil enzyme activities and selected soil parameters (n=8)

Parameter	MBC	OC	*Azospirillum*	PSB	FDA hydrolase	PMEase	DH
MBC	1.000						
OC	0.756*	1.000					
Azospirillum	0.897*	0.811*	1.000				
PSB	0.885*	0.918*	0.877*	1.000			
FDA hydrolase	0.852*	0.809*	0.802*	0.823*	1.000		
PMEase	0.928*	0.829*	0.902*	0.924*	0.785*	1.000	
DH	0.976*	0.754*	0.882*	0.885*	0.845*	0.941*	1.000

*P=0.05

MBC, Microbial biomass carbon; OC, organic carbon; PSB, phosphate-solubilizing bacteria; FDA, fluorescein di-acetate; PMEase, phosphomonoesterase; DH, dehydrogenase

Table 3 Grain yield (pooled means of two years) of rice and wheat and effect of treatments on selected soil chemical[1] characters

Treatment	Crop yield (tonnes/ ha)		Available nutrients status (kg /ha)			Organic carbon	*p*H
	Rice	Wheat	N	P_2O_5	K_2O	(g /kg)	(1:2)
T_1	2.78	0.60	183.98	19.24	131.42	8.65	4.71
T_2	3.55	0.93	223.70	21.91	152.39	9.48	4.57
T_3	3.42	0.81	221.61	22.49	146.39	9.18	4.71
T_4	3.25	0.72	224.76	23.94	138.82	10.03	4.73
T_5	3.47	0.98	234.11	23.44	144.82	9.85	4.96
T_6	3.60	0.72	228.93	24.27	144.98	9.90	4.95
T_7	3.68	0.97	231.00	28.04	136.32	10.38	4.86
T_8	3.08	0.62	224.74	19.24	139.87	8.87	4.87
CD (P=0.05)	0.39	0.07	29.82	2.89	NS	1.00	NS

[1]Means of pooled analysis after the four crops under the sequence

treatment T_7, with substantial slash of chemical fertilizer displayed the greatest PMEase (358.62 µg *p*-nitro phenol/g/hr) activity in soil. This may be attributed to the release of more organic bound P, as synthesis of the enzyme is stimulated by the presence of organic substrate (Biswas and Narayanasamy 2006). The rising MBC, OC accretion ,established *Azospirillum* and PSB populations were extensively correlated with the PMEase activity (Table 2) signifying that compost, biofertilizers and enriched compost plays an important role in protecting and maintaining soil enzymes in their active forms (Saha *et al.* 2008).

DH is an intracellular enzyme of microorganisms and has been used as a parameter to study biological activity of soil. In the present study, DH has been found to increase significantly in soils applied with both the combination of organic and inorganic fertilizer in the crop sequence over no fertilized treatment (Table 1). Substitution of 25% of NP fertilizers either through compost (2tonnes/ha) with biofertilizers or by way of enriched compost (2tonnes/ha) in treatments T_5 and T_7, promoted higher levels of DH activity of 152.94 and 177.11 µgTPF/g/24hr respectively over 100% NPK fertilized plots (64.63 µgTPF/g/24hr). The enriched compost application @2tonnes/ha was found superior in improving DH activity (177.11 µg TPF/g/24hr) as it is stratified microbial populations. Bulky source of potential beneficial microbes in the enriched compost may possibly provide microbial diversity and activity of microorganisms accompanied by better DH activity. Singh and Dhar (2011) also described favourable improvement on the DH activity in organically managed rice–wheat–greengram cropping system. The positive correlation (Table 2) between DH with MBC, OC, *Azospirillum* and PSB population of the study indicate that the improvement of soil quality following environment-friendly nutrient management.

MBC, OC, Azospirillum and PSB populations

Soil MBC, the most active and dynamic pool of the soil organic matter, function as transient nutrients sinks and are responsible for releasing nutrients from organic matter for use by plants. The measurement of MBC after four crops under rice–wheat sequence illustrated significant differences among the INM treatments (Table 1). Of the organic inputs, either integration of compost (2tonnes/ha) with biofertilizers or application of enriched compost (2tonnes/ha) amid 25% NP fertilizer in treatment T_5 and T_7 resulted higher MBC at 167.66 and 183.66 µg/g respectively, whereas the lowest (43.23 µg/g) was obtained from the control plots. Build-up of microbial biomass is mainly due to the microbial biomass contained in the organic residues and the addition of substrate carbon, which stimulates the indigenous soil micro-biota. Likewise, Singh *et al.* (2009a) indicated the integrated roles of organics and chemical fertilizers in increasing MBC under maize-wheat system. Application of biofertilizers, besides showing their primary effect are also known to produce diverse growth promoting substances that might contribute intense proliferation of microbial growth and augmented MBC.

Continuous application of compost, biofertilizers or enriched compost with chemical fertilizers significantly increased the OC of the soil (Table 3). Maximum OC (10.38 g/kg) was observed in the treatment T_7, with the application of enriched compost (2 tonnes/ha) amid sizeable cut of chemical fertilizer after four crops. Compared to control and inorganic fertilized soil, the higher OC under INM treatments might be due to the direct application of carbon input (Singh and Dhar 2011), which could be enhanced further through root exudates, root residue of rice and wheat and biofertilizers application (Singh and Pathak 2003).

Over the period of the experiment, organic amendments along with low quantity of mineral fertilizer appreciably increased *Azospirillum* and PSB population as compared to the mineral fertilizer alone under the sequence (Table 1). Among the INM treatments, the application of compost(2 tonnes/ha) with biofertilizers in treatment T_5, exhibited significantly highest *Azospirillum* (5.79 log cfu/g) count, followed by enriched compost (5.68 log cfu/g) in treatment

T_7 even over the RD of mineral fertilizers (4.90 log cfu/g). With regards to PSB, enriched compost (2 tonnes/ha) in treatment T_7 contributed significantly highest (5.10 log cfu/g) population, followed by compost (2 tonnes/ha) with biofertilizers (4.78 log cfu/g) in treatment T_5. The results illustrated that the greater part of the favourable effects of elevated and reasonably stabilized specific populations of *Azospirillum* and PSB were related to the added micro-organisms as well as application of compost and enriched compost. The increased population of *Azospirillum* and PSB showed significant correlation (Table 2) with MBC, OC and enzyme activities. As a consequence, organic inputs generally enhanced the development of micro flora and increase the global activity of soil.

Grain yields, soil p*H and available nutrients status*

Application of enriched composts, compost and biofertilizers in INM, resulted in a significant increases the grain yield of rice as well as wheat (Table 3). On comparison with recommended dose of chemical fertilizer, the rice yield significantly highest (3.68 tonnes/ha) in the INM treatment T_7, which received 25% NP and coupled with enriched compost (2 tonnes /ha).Though, the overall yield performance of wheat were lower in the INM treatments, highest yield recorded (0.98 tonnes/ha) in the treatment T_5 that contained compost (2 tonnes/ha) with biofertilizers. Integration of enriched compost, compost and biofertilizers with inorganic fertilizers increases the MBC, OC and showed higher soil enzymatic activities that facilitate favourable soil conditions for proper plant growth.

The soil *p*H after the four crops in the present study appeared to be reasonably stable (4.57–4.96) under the treatments (Table 3). Although non-significant, compared to the inorganic fertilized soil (*p*H 4.57), the results illustrated the minor improvement of soil *p*H (4.86–4.96) in the treatments T_5, T_6, T_7 and T_8. These favorable effects of improved *p*H (4.86–4.96) in the treatments (T_5, T_6, T_7 and T_8) could be related to the integrated use of organic inputs reflecting their buffering capacities in acidic soils.

Of the available nutrients estimated, nitrogen was significantly improved (Table 3) under INM treatments due to the application of compost and biofertilizers and significantly highest available N was recorded in the treatment T_5 (234.11 kg/ha), followed by T_7 (231.00 kg/ha).Continuous application of N-fixing biofertilizer agent with organics is attributed to improve soil available N. Similarly, the available P_2O_5 was improved significantly following conjoint use of either compost and biofertilizers or enriched compost even over the recommended doses of chemical fertilizers. Highest available phosphate (28.04 kg/ha) was obtained in the treatment T_7 in enriched compost (2 tonnes/ha) treated plots. RP carrying enriched compost could have augment the available phosphate in the treatments (Singh *et al.* 2009a) signifying release of more organic bound P and ascribed to increased phosphatase activity (Biswas and Narayanasamy 2006). Although not significant, if compared to the initial status of the nutrients however, there were substantial improvements of the available K under the INM treatments.

It may be concluded that the regular use of organic inputs coupled with reduced chemical fertilizer can improve the chemical and biological properties of soil under rice–wheat sequence. In particular, use of enriched compost with low amount of chemical fertilizer improved the soil enzyme activities, microbial biomass carbon, microbial population as well as organic carbon concentration in soil under the sequence.

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